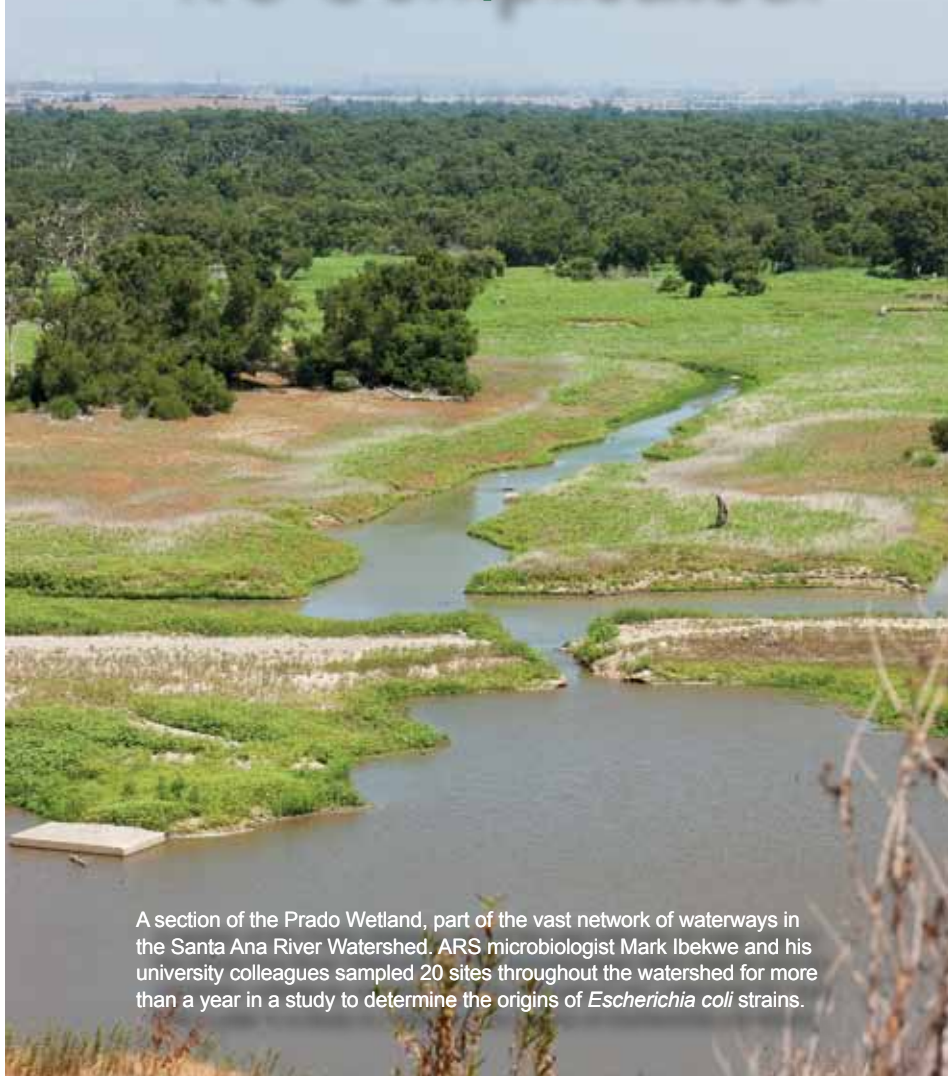


# Where Does *E. coli* Come From?

## It's Complicated!



A section of the Prado Wetland, part of the vast network of waterways in the Santa Ana River Watershed. ARS microbiologist Mark Ibekwe and his university colleagues sampled 20 sites throughout the watershed for more than a year in a study to determine the origins of *Escherichia coli* strains.

**“People blame cows for so many things,”** says Agricultural Research Service scientist Mark Ibekwe.

However, Ibekwe has now identified telltale links between the *Escherichia coli* bacteria living in the streams and sediments of one southern California watershed and their origins—findings that could help restore the reputation of the local livestock. His studies suggest that the pathogens that end up in local waterways are more often carried there via runoff from urban areas, not from animal production facilities.

Public-health officials use *E. coli* as an indicator of water quality, and cows are often seen as the culprits when *E. coli* is found in local lakes, rivers, and other bodies of water. The bacteria can survive in surface water and sediment because of high nutrient content from manure from livestock facilities, runoff from residential areas, warm temperatures, and inputs from other urban sources. Even though most strains of *E. coli* are nonpathogenic, bacterial counts in the Santa Ana River Watershed have exceeded U.S. Environ-

mental Protection Agency water-quality standards.

Ibekwe, who works at the U.S. Salinity Laboratory in Riverside, California, studied hundreds of *E. coli* isolates he collected from the middle Santa Ana River Watershed to determine their source. Since the region contains both concentrated animal-feeding operations and a sizable human population, natural-resource managers are concerned about the diverse number of *E. coli* populations throughout the watershed. “There’s only so much a municipal water treatment plant can do,” Ibekwe notes. “There are 11 water treatment plants that discharge into the middle Santa Ana River in our study area, and that discharge makes up 90 percent of the summer flow.”

### Sourcing Solutions

Ibekwe, California State Polytechnic University researcher Shelton Murinda, and North Carolina State University researcher Alexandria Graves spent more than a year collecting 450 water and sediment samples from 20 sites throughout the watershed. The sites included urban areas, agricultural areas, parks, national forest lands—which provided information about bacterial contributions from wildlife and other background sources in undeveloped areas—and three wastewater treatment plants.

Then the scientists extracted *E. coli* bacteria from each sample and used pulsed-field gel electrophoresis (PFGE) to find distinctive segments of the organism’s DNA that could be used to assign isolates to different groups. These DNA patterns, called “fingerprints,” are detected by separating bacterial DNA into tiny pieces, placing the pieces in a gel, and then sending electricity through the gel. The electrical current separates the DNA pieces according to size to create a banding pattern. This is the fingerprint of each isolate. The individuals can be sorted into different groups, called “clonal populations,” based on their fingerprint similarities.

Using PFGE, the researchers identified 600 different isolates of *E. coli* in their samples, many of which could be placed into 6 clonal populations. They found the greatest variety of different types of *E. coli* in runoff discharged from areas dominated by urban development or human activities.

“I think this is our most important finding—that *E. coli* populations in urban runoff are more genetically diverse than *E. coli* populations in agricultural runoff,” Ibekwe says.

Ibekwe and colleagues also found that *E. coli* isolates collected from water and sediment samples at the same location and at the same time nevertheless exhibited a considerable level of genetic diversity. In addition, water samples often contained a more diverse assortment of *E. coli* isolates than sediment samples. This suggests that the *E. coli* found in sediment samples could belong to permanent population reservoirs, not transient populations that fluctuate along with discharge into the water channel.

#### A Range of Resistance

As part of the study, Ibekwe also tested all the *E. coli* isolates for their resistance to various antibiotics: rifampicin, tetracycline, erythromycin, cephalothin, streptomycin, ampicillin, and amoxicillin clavulanate. He found that between 88 to 95 percent of the isolates were resistant to rifampicin and that around 75 percent were resistant to tetracycline, an antibiotic commonly used to treat a range of infections in humans.

Tetracycline resistance was by far the most common type of resistance observed in *E. coli* isolates collected near wastewater treatment plants. The scientists also found that 24 percent of *E. coli* collected in sediment samples associated with urban runoff—a total of 144 isolates—showed resistance to as many as 7 antibiotics. The antibiotics associated with most multiple resistances were rifampicin, tetracycline, and erythromycin, and the 5 isolates with the highest multiple antibiotic resistance were found in streambed sediments collected from areas that receive urban runoff.

Finally, Ibekwe was surprised when they found 53 *E. coli* isolates that, based on DNA fingerprinting, they could not assign to any of the 6 clonal groups. But like the other isolates, these 53 outliers showed a range of resistance to several antibiotics.

The sheer range of different antibiotic-resistant *E. coli* isolates identified by Ibekwe and his colleagues suggests that public-health officials who track water

quality might need to increase their database of *E. coli* fingerprints. “If we want to use PFGE for source tracking in a large watershed like the Santa Ana River, a very extensive DNA fingerprint library is needed, because our study shows that even a minor change in the DNA fingerprint can significantly affect clonal groupings,” Ibekwe explains. “The fingerprint library will have to include isolates from potential multiple-contaminant sources and isolates that vary over time and space throughout the watershed. This will help in correctly

identifying isolates that are a health concern.”—By **Ann Perry**, ARS.

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Technician Damon Baptista (left) and microbiologist Mark Ibekwe collect a water sample from a creek that drains into the middle Santa Ana River Watershed. They are studying how the thousands of surrounding dairies affect the creek's water quality.

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